A.1.2. Inclusion/exclusion criteria

All types of pesticides, including those banned in the EU, were considered to enhance the totality of the epidemiological evidence available at the time of the review.

Exclusion criteria:

- Studies without control populations (case reports, case series) and ecological studies
- Pesticide poisoning or accidental high dose exposure
- Studies with no quantitative information on effect estimates
- Studies with different follow-up periods and examining the same outcome, only the one with the longest follow-up was retained to avoid data duplication.
- Studies referred to the adverse effects of substances used as therapy for various medical conditions (e.g., warfarin-based anticoagulants)
- Studies on solvents and other non-active ingredients (e.g. co-formulants) in pesticides
- Studies examining the association between exposure and biomarkers of exposure were not considered eligible as they do not examine health outcomes
- Studies/analyses investigating exposure to pesticides: arsenic, hexachlorocyclohexane (HCH) α or β, lead, dioxins and dioxin-like compounds including polychlorinated biphenyls (PCBs) were not considered
- Narrative reviews were excluded but not systematic reviews or meta-analyses

Publications reporting series of acute poisonings or clinical cases, biomonitoring studies unrelated to health effects, or studies conducted on animals or human cell systems were not included; only epidemiological studies addressing human health effects were selected. Publications that lacked quantitative data for measuring associations were also excluded.

Cohort studies, case-control studies and cross-sectional studies were included. Each study underwent an assessment of its eligibility based on a method including 12 criteria such as study design, precise description of the inclusion/exclusion criteria, level of detail in describing exposure, robustness in the measurement of exposure, adjustment for potential confounding factors, method of assessment of the health outcome, sample size, etc. Among these 12 criteria, three were related to the degree of precision in the description/measurement of exposure, which may explain why a large number of epidemiological studies were not selected.

A.1.3. Results

Overall, 602 individual publications were included in the scientific review. These 602 publications corresponded to 6,479 different analyses. The overwhelming majority of evidence comes from retrospective or cross-sectional studies (38 and 32% respectively) and only 30% of studies had a prospective design. Exposure assessment varied widely between studies and overall 46% measured biomarkers of pesticides exposure and another 46% used questionnaires to estimate exposure to pesticides. Almost half of the studies (49%) were based in America. Most studies examined associations between occupational exposure to pesticides and health effects. The entire spectrum of diseases associated with pesticides has not been studies before. The report examined a wide variety of outcomes (Fig. 6). The largest proportion of studies pertains to cancer outcomes (N=164) and outcomes related to child health (N=84).

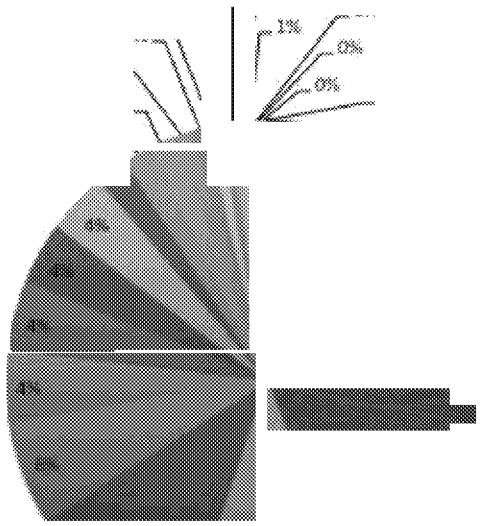

Figure 6: Major outcome categories and corresponding percentage of studies examining those outcomes among the publications reviewed by the EFSA external scientific report (Ntzani et al., 2013).

Despite the large volume of available data and the large number (>6,000) of analyses available, firm conclusions were not made for the majority of the outcomes studied. This was due to several limitations of the data collected as well as to inherent limitations of the review itself. As mentioned above, the review studied the whole range of outcomes examined in relation to pesticides during 5 years' period. Thus, only recent evidence was reviewed and the results of the meta-analyses performed should be cautiously interpreted as they do not include all the available evidence. It is therefore capable of highlighting outcomes which merit further in-depth analysis in relation to pesticides by looking at the entire literature (beyond 5 years) and by focusing on appraising the credibility of evidence selected. The limitations of the studies itself are in line with other filed of environmental epidemiology and focus around the exposure assessment, the study design, the statistical analysis and reporting. In particular:

a) **Exposure assessment**: The assessment of exposure is perhaps the most important methodological limitation of the studies reviewed in the ESR. Studies used different methods for exposure assessment and assignment. Most studies were based on self-reported exposure to pesticides, defined as "ever versus never" use or as "regular versus non-regular" use. Such methods suffer from high misclassification rates and do not allow for dose response analysis. This is especially the case for retrospective studies where misclassification would be differential with higher exposures reported in participants with disease (recall bias) (Raphael, 1987). While questionnaires might be capable of differentiating subjects with very high and very low exposure levels, they are not capable of valid exposure classification across an exposure gradient, thus not allowing the study of dose-response relationships. Also, questionnaire for exposure assessment need to be validated for use in epidemiological studies. Nonetheless, a vast proportion of studies use in house version of non-validated questionnaires which may suffer from content (the questionnaire does not cover all sources

of exposure to the hazard of interest) or criterion validity (e.g., through inaccurate recall or misunderstanding of questions) (Coggon, 1995).

Although the range of categories of pesticide studied is wide, studies very often concentrate on a broadly defined pesticide category, so that it is difficult to know what type of pesticide the population is exposed to.

Exposure to pesticides was defined as reported use of pesticides by the study participant or by government registry data. These derive from self-administered questionnaires, interviewer administrated questionnaires, job exposure matrices (JEM), by residential status (proximity to pesticide exposure), by detecting biomarkers associated with pesticide exposure or by other means as defined by each study.

Studies often examine pesticides that have already been banned in western populations and the EU. The use of biomarkers as means of exposure assessment is infrequent, but still available in almost half of the studies.

b) **Study design**: As mentioned above, the majority of evidence comes form case-control studies and cross-sectional studies. Cross-sectional, and in part also case-control studies, cannot fully assess the temporal relationships and thus are less able to provide support regarding the causality of associations.

c) **Outcomes examined**: The definition of clinical outcomes displayed large variability in eligible epidemiological studies, which can further cause the variability in results. Perhaps most important in this setting is the use of a great number of surrogate outcomes examined. Surrogate outcomes are biomarkers or physical measures that are generally accepted as substitutes for, or predictors of, specific clinical outcomes. However, often these surrogate outcomes are not validated and do not meet the strict definitions of surrogate outcomes. Such outcomes can be defined as possible predictors of clinical outcomes but do not fulfil the criteria for a surrogate outcome. It is essential to appraise the evidence around non-validated surrogate outcomes by taking into account the implicit assumptions of these outcomes.

A great variety of assessed outcomes covering a wide range of pathophysiologies was observed. "Hard" clinical outcomes as well as many surrogate outcomes included in the database reflect the different methodologies endorsed to approach the assessed clinical research questions. The different outcomes were divided into 23 major disease categories, with the largest proportion of studies addressing cancer and child health outcomes.

The adverse health effects assessed included:

a) major clinical outcomes, such as cancer, respiratory (allergy), reproductive (decreased fertility, birth defects) and neurodegenerative (Parkinson's disease);

b) clinical surrogate outcomes, e.g. neurodevelopmental impairment (assessed by neurocognitive scales) and

c) laboratory surrogate outcomes (e.g., liver enzyme changes).

For many adverse health effects attributed to pesticide exposure there exist contradictory or ambiguous studies. Whether this results from lack of consistency or real heterogeneity warrants further clarification.

d) **Statistical analysis**:

Simultaneous exposure to multiple agents (heavy metals, solvents, suspended particulate matter etc.) from different sources is common. It may introduce further bias in the results as all of them may produce adverse health outcomes. Thus, it is essential to account for confounding from exposure to multiple agents in order to delineate true associations but this has not been possible in the overwhelming majority of evidence assessed in the EFSA external scientific report.

In addition, the evidence collected and appraised in the EFSA external scientific report (Ntzani et al., 2013) is likely to suffer from selective reporting and multiple testing. The studies reported a very wide range of analyses; 602 publications resulted in 6000 analyses. The amount of multiple hypothesis testing is enormous. These analyses need to be adjusted for multiple hypothesis testing else, otherwise the results suffer from high false positive rate. Even when studies present only one analysis, selective reporting is always a possibility as has been shown in other epidemiological fields as well. In addition, when interpreting results one should also take into account that, especially for certain outcomes (e.g. cancers), the majority of evidence comes from single study populations and the Agriculture Health Study in particular.

A.1.4. Conclusion of the EFSA External Scientific Report

Regardless of the limitations highlighted above, the External Scientific Report (Ntzani et al., 2013) showed consistent evidence of a link between exposure to pesticides and Parkinson's disease and childhood leukaemia, which was also supported by previous meta-analyses. In addition, an increased risk was also found for diverse health outcomes less well studied to date, such as liver cancer, breast cancer and type II diabetes. Effects on other outcomes, such as endocrine disorders, asthma and allergies, diabetes and obesity showed increased risks and should be explored further.

Childhood leukaemia and Parkinson's disease are the two outcomes for which a meta-analysis after 2006 was found consistently showing an increased risk associated with pesticide exposure. Nonetheless, the exposure needs to be better studied to disentangle the effect of specific pesticide classes or even individual pesticides. Significant summary estimates have also been reported for other outcomes (summarised in Table 4). However, as they represent studies from 2006 onwards results should be regarded as suggestive of associations only and limitations especially regarding the heterogeneity of exposure should always been taken into consideration. Data synthesis and statistical tools should be applied to these data in relation to specific outcomes, after the update of the results to include publications before 2006, in order to quantify the amount of bias that could exist and isolate outcomes where the association with pesticides is well supported even when estimates of bias are taken into account. Similarly, outcomes where further evidence is needed to draw firm conclusions need to be highlighted.

Table 4: Summary of meta-analyses performed in the report.

Health outcome	N studies	Meta-analysis results	I^2
Leukaemia	6	1.26 (0.93; 1.71)	59.4%
Hodgkin lymphoma	7	1.29 (0.81-2.06)	81.6%
Childhood leukaemia (exposure to pesticides during pregnancy)	6	1.67 (1.25-2.23)	81.2%
Childhood leukaemia (exposure to insecticides during pregnancy)	5	1.55 (1.14-2.11)	65%
Childhood leukaemia (exposure to insecticides during pregnancy – update Turner, 2010)	9	1.69 (1.35-2.11)	49.8%
Childhood leukaemia (exposure to unspecified pesticides during pregnancy)	5	2.00 (1.73-2.30)	39.6%
Childhood leukaemia (exposure to unspecified pesticides during pregnancy – update Turner, 2010)	11	1.30 (1.06-1.26)	26.5%
Childhood leukaemia (exposure to pesticides during childhood)	7	1.27 (0.96-1.69)	61.1%
Childhood leukaemia (exposure to insecticides during childhood – update Turner, 2010)	8	1.51 (1.28-1.78)	0%
Childhood leukaemia (exposure to unspecified	11	1.36 (1.19-1.55)	0%

pesticides during childhood – update Turner, 2010)			
Breast cancer (DDE exposure)	5	1.13 (0.81-1.57)	0%
Breast cancer	11	1.24 (1.08-1.43)	0%
Testicular cancer (DDE exposure)	5	1.40 (0.82-2.39)	59.5%
Stomach cancer	6	1.79 (1.30-2.47)	0%
Liver cancer		2.50 (1.57-3.98)	25.4%
Cryptorchidism	85	1.19 (0.96-1.49)	23.9%
Cryptorchidism (DDT exposure)	4	1.47 (0.98-2.20)	51%
Hypospadias (general pesticide exposure)	6	1.01 (0.74-1.39)	71.5%
Hypospadias (exposure to specific pesticides)	9	1.00 (0.84-1.18)	65.9%
Abortion	6	1.52 (1.09-2.13)	63.1%
Parkinson's disease	26	1.49 (1.28-1.73)	54.6%
Parkinson's disease (DDT exposure)	5	1.01 (0.78-1.30)	0%
Parkinson's disease (paraquat exposure)	9	1.32 (1.09-1.60)	34.1%
Amyotrophic lateral sclerosis	6	1.58 (1.31-1.90)	10%
Asthma (DDT exposure)		1.29 (1.14-1.45)	0%
Asthma (paraquat exposure)	65	1.40 (0.95-2.06)	53.3%
Asthma (chlorpyrifos exposure)	5	1.03 (0.82-1.28)	0%
Type 1 diabetes (DDE exposure)	8	1.89 (1.25-2.86)	49%
Type 1 diabetes (DDT exposure)	6	1.76 (1.20-2.59)	76.3%
Type 2 diabetes (DDE exposure)	4	1.29 (1.13-1.48)	0%

N=number of studies considered for the meta-analysis; in the column of meta-analysis results the numbers represent the statistical estimate for the size of effect (odds ratio –OR–, or Relative Risk – RR–) with the corresponding 95% confidence interval (CI). I^2 represents the percentage of total variation across studies that is due to heterogeneity.

A.2. The INSERM report

In September 2013, the French National Institute of Health and Medical Research (INSERM) released a literature review carried out with a group of experts on the human health effects of exposure to pesticides[20]. Epidemiological or experimental data published in the scientific literature up to June 2012 were analysed. The report was accompanied by a summary outlining the literature analysis and highlighting the main findings and policy lines, as well as the recommendations.

The INSERM report is composed of four parts: 1) exposure assessment, with a detailed description of direct and indirect methods to assess exposure in epidemiological studies; 2) epidemiology, with an inventory and analysis of epidemiological studies available in the literature up to 2012, and a scoring system to assess the strength of presumed association; 3) toxicology, with a review of toxicological data (metabolism, mode of action and molecular pathway) of some substances and assessment of biological plausibility; and 4) recommendations.

The vast majority of substances identified by the INSERM report as having a presumed moderate or strong association with the occurrence of health effects are chemicals that are now prohibited. This is mainly driven by the fact that the majority of the diseases examined are diseases of the elderly; therefore, the studies performed to date are based on persons who were old at the time of the study and exposed many years ago. By definition, it is not yet possible to investigate the potential long term effects of many of the more recent products.

These substances belong to the group of organochlorine insecticides, such as DDT or toxaphene, or insecticides with cholinesterase-inhibiting properties, such as terbufos or propoxur.

[20] INSERM. Pesticides. Effets sur la santé. Collection expertise collective, Inserm, Paris, 2013